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Effect of physicochemical parameters on the retention of some monoamine oxidase inhibitory drugs on a porous graphitized carbon column

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Abstract

The retention of sixteen monoamine oxidase inhibitory drugs (proparlgylamine derivatives) was determined on a porous graphitized carbon (PGC) column using ethanol–water mixtures as eluents. The HPLC retention characteristics of drugs were correlated with their physicochemical properties using stepwise regression analysis and principal component (PC) analysis. The dimensions of the matrices for PC loadings and variables was reduced using a non-linear mapping technique, varimax rotation and cluster analysis. It has been established that the drugs can be well separated on the PGC column in ethanol–water eluents. Calculations proved that the retention behavior of monoamine oxidase drugs on PGC column is of mixed character: both steric and electronic parameters influence the retention.

Keywords: Porous graphitized carbon columns; Monoamine oxidase

1. Introduction

A porous graphitic carbon (PGC) support has been developed in the last decade [1,2]. The development of this highly pH-stable type of column was motivated by the fact that the use of silica or silica-based supports in high-performance liquid chromatography (HPLC) is limited by the low stability of silica at high pH values [3] and, in the case of reversed-phase silica-based supports, by the undesirable electrostatic interactions that occur between the polar substructures of solutes and the free silanol groups not covered by the hydrophobic ligand [4]. PGC columns have been successfully used for the separation of diastereoisomers [5], geometric isomers [6], and various bioactive compounds such as taxol [7], neuroleptic agents [8], anti-hypoxia drugs [9], etc. The effect of various physicochemical parameters of solutes on their retention behaviour on PGC columns has been studied in detail and the importance of steric and electronic interactions between solutes and the stationary phase has been emphasized [10]. As the logarithm of the capacity factor generally depends linearly on the concentration of the stronger component in the eluent, this relationship has been used frequently for the determination of the retention capacity (intercept of the correlation) and for the calculation of the specific surface area of solutes in contact with the support (slope of the correlation) [11]. It was assumed that the significant correlation between the intercept and slope values of a set of solutes indicates their structural homogeneity [12].

Multivariate mathematical statistical methods, such as principal component analysis (PCA) [13] and

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cluster analysis (CA) [14], have been developed to extract maximum information from large data matrices. PCA and CA have also been used sucessfully for the evaluation of data structure in various fields of chromatography such as thin-layer chromatography (TLC) [15], gas chromatography [16] and HPLC [17]. The advantages of the application of PCA in chromatography is that it allows a reduction in the number of variables whilst maintaining most of the information content.

Propargylamine derivatives are selective inhibitors of B-type monoamine oxidase (MAO) [18]. Their mode of action has not been clarified in detail. However, it was etablished that they readily interact with dicarboxy amino acids [19] and with membrane phospholipids [20].

The objectives of our investigation was to study the retention behaviour of monoamine oxidase inhibitory drugs on a PGC column, to elucidate the relationship between the retention characteristics and the measured and calculated physicochemical parameters of drugs, by means of multivariate mathematical statistical methods, and to compare the performance of these methods for the evaluation of chromatographic retention data matrices.

2. Experimental

The chemical structures of monoamine oxidase inhibitory drugs are compiled in Table 1. The PGC column (Shandon Hypercarb 100 × 4.7 mm I.D., particle diameter 7 μm) was purchased from Shandon Scientific (Runcorn, UK). The HPLC equipment consisted of a Gilson gradient analytical system from Gilson Medical Electronics (Villiers-le-Bell, France) with 2 piston pumps (Model 302), a detector (Model 116), a Rheodyne injector with a 20-μl sample loop (Cotati, CA, USA), and a Waters 740 integrator (Milford, MA, USA). The flow-rate was 1.0 ml/min and the detection wavelength was 240 nm. Mixtures of ethanol–water were used as eluents. The ethanol concentration varied between 75–95 vol.% in steps of 5 vol.%. The drugs were dissolved in the eluents at a concentration of 0.05 mg/ml. The retention time of each compound, in each eluent, was determined by three consecutive determinations. In order to determine the retention capacity and the specific contact surface of the drugs, linear correlations were calculated between the log k value of the drugs and the ethanol concentration in the eluent:

$$\log k = \log k_0 + b \cdot C \quad (1)$$

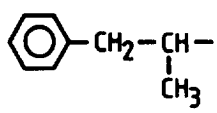
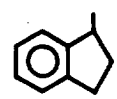
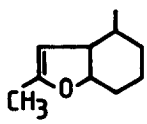
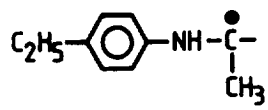
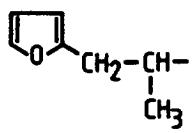
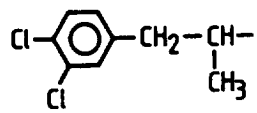
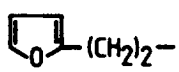
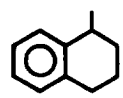
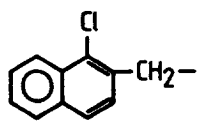
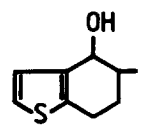
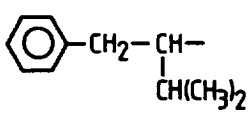
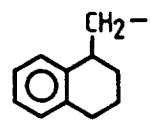
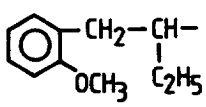
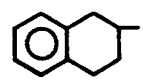
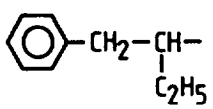
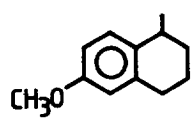
where: log k = logarithm of the capacity factor; log k_0 = logarithm of the capacity factor extrapolated to zero ethanol concentration in the mobile phase (intercept), b = change of log k value caused by a unit change (1 vol.%) of ethanol concentration in the eluent (slope) and C = ethanol concentration (vol.%) in the eluent. The intercept and slope values were considered as the best estimation of the retention capacity and of the specific surface area of the drugs in contact with the stationary phase.

To test the validity of the hypothesis that, in the case of an homologous series of compounds, the slope and intercept values of Eq. 1 are intercorrelated, the linear correlation was calculated between the two retention parameters.

The relationship between the retention behaviour of MAO inhibitory drugs and their calculated physicochemical parameters was evaluated by PCA, combined with varimax rotation, cluster analysis and with non-linear mapping [21]. The parameters of Eq. 1 (slope and intercept values) and various calculated physicochemical parameters of drugs (altogether twelve parameters) were considered as variables and the drugs were the observations. The limit of the variance explained was set to 99.9%. The physicochemical parameters included in the calculation were: π = Hansch–Fujita's substituent constant characterizing hydrophobicity [22,23]; *H-Ac* and *H-Do* = indicator variables for proton acceptor and proton donor properties, respectively [24]; *M-RE* = molar refractivity [25]; F and R = electronic parameters characterizing the inductive and resonance effect, respectively [26]; σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent [27]; *Es* = Taft's constant, characterizing the steric effects of the substituent [28]; B_1 and B_4 = Sterimol width parameters determined by the distance of the substituents at their maximum point perpendicular to attachement [29,30]. The physicochemical parameters were calculated from the fragmental contants given in Ref. [24], considering the additivity rule to be valid. The calculated physicochemical parameters of MAO inhibitory drugs are

Table 1
Chemical structures of MAO inhibitory drugs

General structure $R_1-N(R_2)-CH_2-C\equiv CH$

Compound	R_1	R_2	Compound	R_1	R_2
1.		$-CH_3$	9.		$-CH_3$
2.		$-CH_3$	10.		$-H$
3.		$-H$	11.		$-CH_3$
4.		$-CH_3$	12.		$-CH_3$
5.		$-CH_3$	13.		$-CH_3$
6.		$-CH_3$	14.		$-CH_3$
7.		$-CH_3$	15.		$-CH_3$
8.		$-C_4H_7$	16.		$-CH_3$

compiled in Table 2. Varimax rotation around two axes and two-dimensional non-linear mapping were carried out on the PC loadings and variables. To elucidate the influence of PCA, the data structure cluster analysis was applied to the original data matrix. Varimax rotation and the non-linear mapping technique are theoretically similar: each method reduces the dimensions of multidimensional data matrices, with the lowest possible loss of information. As the calculation method of varimax rotation and non-linear mapping is different, we tried to compare their information content by calculating linear correlations between the corresponding distances:

Table 2
Physicochemical parameters of MAO inhibitory drugs calculated according to the additivity rule

Compound	Physicochemical parameter									
	π	*H-AC*	*H-DO*	*M-RE*	*F*	*R*	σ	*Es*	B_1	B_4
1	4.02	0	0	46.07	0.02	−0.15	−0.08	−6.66	4.74	8.54
2	4.40	1	0	56.49	0.07	−1.05	−0.24	−6.02	7.43	14.74
3	2.53	1	0	36.62	0.19	−0.63	0.03	−2.60	2.87	8.20
4	2.62	1	0	37.61	0.14	−0.78	−0.04	−1.55	4.39	9.80
5	5.75	0	0	68.05	0.49	−0.57	0.35	−11.09	8.24	12.10
6	5.19	0	0	55.26	−0.02	0.29	−0.17	−6.70	4.74	10.09
7	4.54	1	0	59.48	0.15	−0.92	−0.03	−8.23	7.61	12.99
8	6.13	0	0	65.57	−0.08	−0.36	−0.16	−8.07	6.26	13.47
9	4.66	0	0	53.03	0.13	−0.19	−0.06	−6.81	5.26	9.13
10	2.15	1	1	50.63	0.24	−0.81	0.04	−6.44	6.32	9.52
11	4.93	0	0	52.38	0.88	−0.54	0.73	−7.36	6.82	10.12
12	5.03	0	0	56.70	−0.13	−0.19	−0.06	−7.00	5.26	8.64
13	2.75	1	1	44.76	−0.40	−1.22	−0.45	11.31	6.77	10.98
14	5.59	0	0	63.35	−0.17	−0.32	−0.30	−8.33	6.78	10.68
15	5.03	0	0	57.70	−0.13	−0.19	−0.23	−7.09	5.26	8.64
16	5.01	0	0	65.57	0.13	−0.70	−0.11	−7.64	6.61	11.51

$$Y_{1,2} = a + b \cdot X_{1,2} \quad (2)$$

where $Y_{1,2}$=first and second coordinates of the nonlinear map; $X_{1,2}$=first and second coordinates of the varimax rotation.

Stepwise regression analysis [31] was used to find the relationship between the retention behaviour of drugs on a PGC column and the various hydrophobic and hydrophilic molecular parameters determined by adsorptive and reversed-phase TLC [32]. In the traditional multivariate regression analysis, the presence of independent variables (in our case chromatographic parameters) that exert no significant influence on the dependent variable (retention behaviour) lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis automatically eliminates, from selected equations, the insignificant independent variables, thus increasing the information power of the calculation.

3. Results and discussion

Each drug had a narrow and symmetrical peak in each eluent proving the good chromatographic characteristics of the PGC column (Fig. 1). The

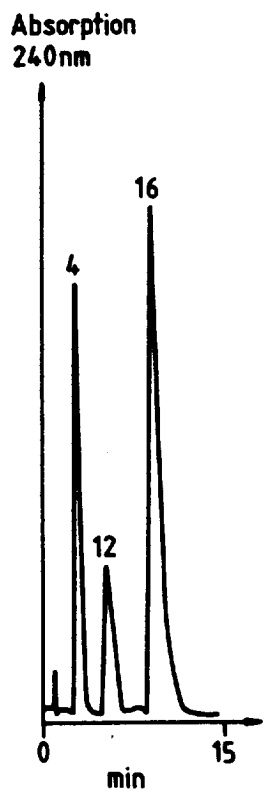


Fig. 1. Separation of some MAO inhibitory drugs on a PGC column. The eluent used was ethanol–water (80:20, v/v), with a flow-rate of 1 ml/min and a detection wavelength of 240 nm. Numbers refer to the drugs listed in Table 1.

Table 3
Parameters of the linear correlations between the log k value of MAO inhibitory drugs and the concentration of ethanol [C(%), v/v] in the eluent

log k = log k_0 + $b \cdot C$				
Compound[a]	log k_0	$-b \cdot 10^2$	$S_b \cdot 10^{3}$ [b]	r
1	3.21	4.09	9.71	0.9949
2	3.27	3.43	1.18	0.9975
3	2.31	2.95	4.62	0.9943
4	3.69	4.73	1.02	0.9931
5	1.54	1.92	4.86	0.9858
6	9.38	11.59	2.40	0.9974
7	4.79	5.57	1.63	0.9948
8	4.90	5.43	1.11	0.9996
9	6.70	7.49	1.67	0.9976
10	4.25	5.50	0.83	0.9977
11	6.34	7.03	2.15	0.9929
12	4.34	4.82	1.41	0.9996
13	3.65	4.18	1.42	0.9988
14	3.51	4.14	7.23	0.9798
15	2.61	2.72	1.23	0.9989
16	4.03	4.30	8.40	0.9945

[a] Numbers refer to the drugs listed in Table 1.
[b] S_b is the standard deviation of the slope (b).

parameters of Eq. 1 are compiled in Table 3. The relationship between the logarithm of the capacity factor and the concentration of the organic phase in the eluent was linear in each instance, the significance level being over 95%. The retention of MAO inhibitory drugs decreases linearly with increasing concentration of the organic component in the eluent, indicating that the drugs also follow the general rule on the PGC column. The slope and intercept values differ from each other, which means that any pair of these drugs can be easily separated on the PGC column using ethanol–water eluents. Furthermore, the parameters compiled in Table 3 make it possible to calculate the retention time differences for each pair of MAO inhibitory drugs at each eluent composition:

$$t_1 - t_2 = t_0(10^{a1+b1C} - 10^{a2+b2C}) \quad (3)$$

where a and b are intercept and slope values for compounds 1 and 2 at an organic phase concentration C.

Significant linear correlation was found between the slope and intercept values of Eq. 1, indicating that the drugs can be considered as an homologous series of solutes on a PGC column (Fig. 2).

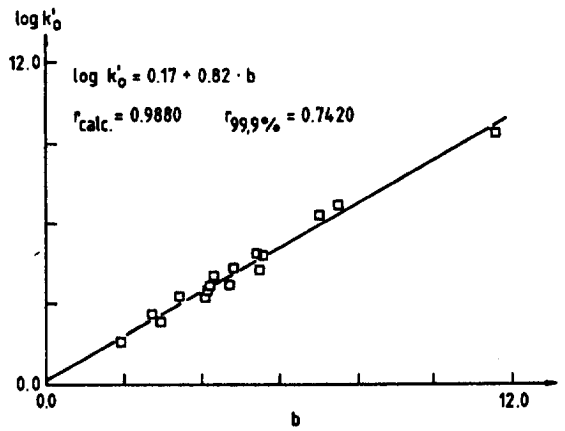


Fig. 2. Relationship between the retention capacity (log k_0) and the specific surface area in contact with the support (b) of MAO inhibitory drugs on a PGC column.

The results of PCA are summarized in Table 4. Four PCs explain more than 90% of the total variance. This result indicates that the twelve physicochemical and chromatographic parameters can be substituted by four background variables, without a substantial loss of information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities, but only indicates their mathematical possibility. However, the parameters having high loadings in a PC can be considered as the concrete physicochemical constituents of the component. Thus, the chromatographic parameters have the highest loadings in the first PC, together with more than one calculated physicochemical parameter. This finding suggests that the retention of drugs on PGC columns is of mixed character and includes both steric and electronic parameters.

The chromatographic parameters did not form a well-defined cluster with the physicochemical parameters on the two-dimensional non-linear map of PC loadings (Fig. 3). Section 2 This result supports our previous conclusions that the retention mechanism of drugs on PGC columns is a complex one, and that more than one physicochemical parameter is involved in the drug–support interaction. Drugs with a condensed ring structure formed a loose cluster on the two-dimensional non-linear map of PC variables, suggesting the involvement of stacking interactions in the retention (Fig. 4).

Table 4
Similarities and differences between the calculated physicochemical parameters of MAO inhibitory drugs and their retention on a PGC column. Results of principal component analysis

Component	Eigen value	Variance explained (%)	Sum of variance explained (%)
1	4.60	38.36	38.36
2	2.75	22.91	61.27
3	2.15	17.96	79.23
4	1.37	11.43	90.65

Parameters	Principal component loadings			
	1	2	3	4
π	0.86	−0.19	−0.42	−0.06
H-Do	−0.71	0.53	0.12	−0.18
H-Ac	−0.50	0.55	0.06	0.51
M-RE	0.82	0.19	−0.47	0.03
F	0.44	0.18	0.76	−0.41
R	0.44	−0.80	0.01	0.31
σ	0.47	0.10	0.80	−0.27
Es	−0.82	0.22	−0.09	0.20
B_1	0.47	0.74	−0.30	−0.15
B_4	0.31	0.66	−0.48	−0.35
log k'_0	0.65	0.43	0.27	0.53
b	0.63	0.41	0.31	0.52

Cluster analysis gave similar, but not identical, results to those of the non-linear mapping technique (Fig. 5 and Fig. 6). However, we advocate the use of the two-dimensional non-linear mapping technique instead of cluster analysis, because the visual evaluation of the similarities between the variables is easier to see on the two-dimensional plane of non-linear mapping than on the one dimensional cluster dendogram. Furthermore, we assume that the two-dimensional non-linear map may contain more information than the one-dimensional stucture of clusters.

Significant linear correlation (r = 0.9876) was found between the first coordinates of non-linear

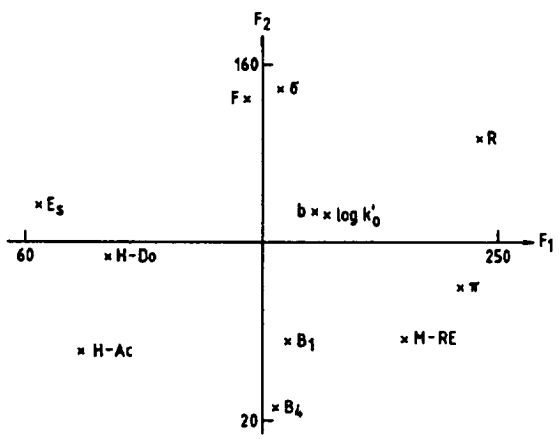


Fig. 3. Similarities and differences between the retention parameters and physicochemical characteristics of MAO inhibitory drugs obtained using a two-dimensional non-linear map of the PC loadings. Number of iterations = 106; maximum error = $4.2.10^{-3}$. For symbols see Section 2.

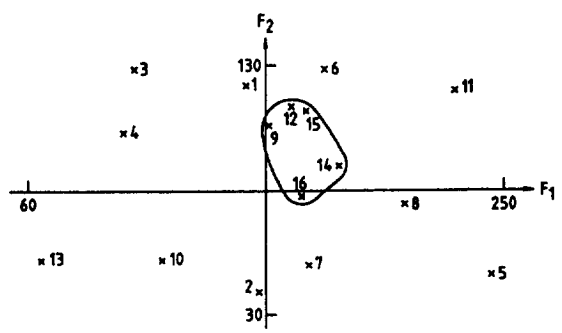


Fig. 4. Similarities and differences between the MAO inhibitory drugs obtained using a two-dimensional non-linear map of the PC variables. Number of iterations = 89; maximum error = $1.2{\cdot}10^{-3}$. Numbers refer to the MAO inhibitory drugs listed in Table 1.

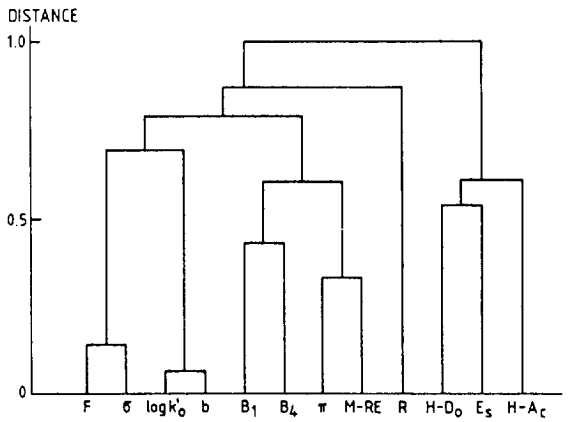


Fig. 5. Similarities and differences between the retention parameters and physicochemical characteristics of MAO inhibitory drugs obtained using a cluster dendogram calculated from the PC loadings. For symbols see Section 2.

mapping and varimax rotation, suggesting the similarity of these methods and indicating that both methods can be used successfully for the reduction of the dimensions of large retention data matrices.

Using stepwise regression analysis, a significant correlation was found between the retention capacity (log k_0) of drugs on the PGC column, their adsorption capacity on silica (X_1) and their specific hydrophilic surface area on alumina surface (X_2) (both of them taken from Ref. [32]):

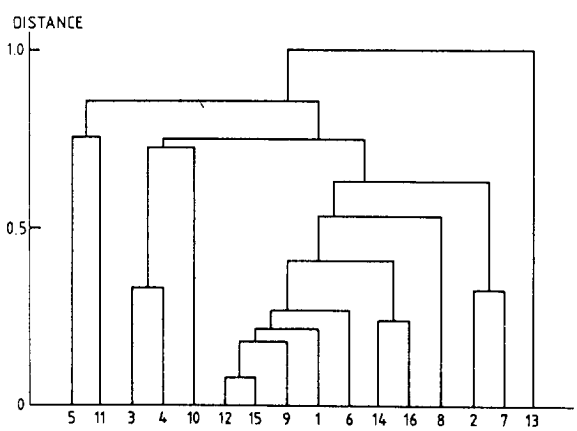


Fig. 6. Similarities and differences between the MAO inhibitory drugs. The cluster dendogram was calculated from the PC variables. Numbers refer to the MAO inhibitory drugs listed in Table 1.

$$\log k_0 = -2.19 + (9.14 \pm 1.62) \cdot X_1 - (6.12 \pm 2.06) \cdot 10^{-2} \cdot X_2 \quad (4)$$

$n = 16$, $r^2 = 0.7100$, $F_{calc.} = 15.91$, $F_{99.9\%} = 12.31$

The significant relationship between the retention capacity of the PGC and of alumina and silica can be explained by the supposition that chromatographic characteristics of PGC show some similarity with those of silica and alumina. However, the ratio of variance explained is only 71%, indicating that marked differences also exist between the retention behaviours. The path coefficients (X_1 = 65.48%; X_2 = 34.52%) indicated that the silica surface shows higher similarity with PGC than the alumina does.

References

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